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Pharmacokinetic study of a coumarin extracted of *Zanthoxylum tingoassuiba* in two animals models

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6-methoxyseselin is a coumarin present in the roots of *Zanthoxylum tingoassuiba* and has many pharmacological properties, such as antinociceptive, anti-inflammatory, and immunomodulatory activities [1, 2]. These effects make it a potential drug candidate. Thus, a preclinical pharmacokinetic study becomes indispensable to evaluate the behavior of this substance in biological systems. Two studies were performed in rats and mice to evaluate the pharmacokinetic parameters, following a previously validated bioanalytical method. In rats (n=3), an intravenous dose of 15 mg/kg was administered, and in mice (n=40), the inhalation and intravenous routes were used with doses of 50 and 15 mg/kg, respectively. Blood samples were collected at the times 0.083, 0.25, 0.5, 0.75 (only in mice), 1, 1.5, 2, 4, 6, 8 and 10 (only in rats) hours after the administration. The samples were treated and analyzed by High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD). The bioanalytical method shows good linearity (r^2 >0.99) and stability for up to 30 days. The PK parameters were estimated by PKAnalix® (Lixoft, France). The values of elimination constant, half-life, clearance, the volume of distribution, and mean residence time were, respectively: 0.41 h^{-1}, 1.7 h, 3.9 L/h/kg, 9.6 L/kg, and 2.29 h. In mice, the values were: 0.3 h^{-1}, 2.31 h, 9.47 L/h, 3.16 L and 2.31 h (intravenous); 0.23 h^{-1}, 2.99 h, 1.94 L/h, 8.36 L and 4.66 h (inhalation). Most parameters did not change significantly between the administration route or animal models, except for the volume of distribution. To our knowledge, this is the first work to study the pharmacokinetics of 6-methoxyseselin in rats and mice.

Ethical approval

All procedures were reviewed and approved by the Committee on Ethics in the Use of Animals (CEUA-IGM) of Oswaldo Cruz Foundation (Fiocruz/ BA) (013-2021).

Financial support and acknowledgements

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Population pharmacokinetic modeling of carbamazepine in patients at a university hospital

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Carbamazepine (CBZ) treats epilepsy and other neurological and psychiatric disorders [1]. Therapeutic monitoring can monitor drugs whose doses are not directly related to plasma concentrations, such as CBZ. Pharmacometrics tools have been used in this monitoring through pharmacokinetic and pharmacodynamic modeling and simulations, such as population pharmacokinetic modeling (PopPK). To analyze concentrations at the sites of action and, when necessary, adjust the dosage [2]. The aim is to develop the PopPK model for patients admitted to the University Hospital Teacher Edgard Santos (HUPES) and used CBZ. Data have been extracted from electronic medical records between 2012 and 2016, using plasma concentrations quantified in HUPES. The Microsoft Office Excel resource was used for statistical analysis, with the ANOVA test followed by Student's t-test comparing the data ratios. Values of $p<0.05$ were considered statistically significant. We obtained 50 patients treated with CBZ, ages 7 to 87 years. Data were divided into subgroups based on the results: underdose, overdose, and normal dose, concerning the therapeutic window. Where among the patients using CBZ, 20 patients (40%) had a normal concentration (>4ug/mL and <10ug/mL), 17 patients (34%) had an underdose and 13 patients (26%) had an overdose. We can reaffirm the importance of CBZ quantification and monitoring with the initial results. A population pharmacokinetic model will be developed to evaluate the data using the software Monolix® (Lixoft, France).

Ethical approval

Research Ethics Committee of the Professor Edgar Santos University Hospital/ UFBA - N° 2.780.692.

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Comparison of Wagner-Nelson and mechanistic deconvolution Methods: application of IVIVR for lamotrigine IR

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In vitro-in vivo relationship (IVIVR) can be a key factor for the earlier successful along the formulation development phase and in future stages of the post-registration of generic drugs [1]. For the construction of an IVIVR, *in vitro* data from dissolution profiles and *in vivo* data from plasma profiles are required. From the plasma concentration data, the percentage of the fraction absorbed or dissolved *in vivo* can be determined, this technique is called deconvolution [2]. Thus, the aim of this work was to compare traditional Wagner-Nelson and Mechanistic deconvolution forms and establish an IVIVR for lamotrigine (LTG) IR, an antiepileptic drug and class 2 in the Biopharmaceutical Classification System (BCS). The dissolution data were obtained from flow-through cell apparatus using biorelevant medium. An oral PBPK was developed and verified using GastroPlus® software. Data deconvolution and IVIVR were performed using the IVIVCPlus® module. The IVIVR models were analyzed by the slope of the regression line, the determination coefficient (Rsq), and visually. The models were validated internally and externally. Mean percentage prediction error (PE%) of a maximum of 15% was accepted [3]. The verifications for both methods showed PE% below 15%. However, the mechanistic absorption method presents smaller errors than the Wagner-Nelson. Although the Wagner-Nelson method is more traditional, the mechanistic method was able to predict *in vivo* dissolution data in a pure and more accurate way. So, it can be the method chosen for other applications of LTG IVIVR as to develop new formulations and to establish a virtual bioequivalence.

Financial support and acknowledgements

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Adult and pediatric physiologically based biopharmaceutics model to explain lamotrigine immediate release dissolution and absorption process

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Physiologically based biopharmaceutics modeling (PBBM) is a viable tool to identify formulation-physiology interactions and predict the clinical impact of variations in formulation parameters [1]. PBBM has an immense potential benefit in speeding up the development of new drugs, ensuring quality, and reducing unnecessary animal and human testing, particularly in children where data is lacking due to ethical issues [2]. Thus, the aim of this work was to develop and verify an adult PBBM model for lamotrigine (LTG) and extrapolate to pediatrics to discuss the impact of dissolution on LTG pharmacokinetics (PK). The dissolution data were obtained from flow-through cell apparatus using biorelevant medium. An oral physiologically based pharmacokinetic model (PBPK) was developed using GastroPlus™ software. The predicted and observed PK profiles were compared to assess the predictability of the model and PBBM model was developed by incorporating dissolution data by calculation of the z-factor. LTG PBPK profiles showed percentage prediction error (PE%) less than 25%. The PBBM model, with dissolution data incorporated, improve the adult PK prediction with tmax %PE from 52% to 13%. For pediatric model, it was necessary to adjust the volume of distribution by optimizing the log P and it was assumed the fed state given the specific characteristics of this population. Employing pediatric dissolution condition, the %PE for AUC, Cmax and tmax were between -12.68-27.07%. So, the dissolution process seems to be an important step for LTG PK. Additionally, the LTG PBBM model can be used in formulation development studies.

Financial support and acknowledgements

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Validation of methodology for quantification of mefloquine for PK/PD modeling

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Malaria is an acute febrile infectious disease caused by intracellular protozoa of the genus *Plasmodium*, which, despite being treatable and preventable, still generates a high mortality rate in the world [1]. Mefloquine is one of the antimalarials used in association for its treatment. However, despite the advantages described, there are still limitations in its use, mainly because of the parameters that guide the dosage. That said, the first step in performing PK/PD modeling is validating a methodology for quantifying mefloquine in plasma, microdialysate, and culture medium. Validation took place according to FDA [2] requirements, using a C18 column (5 µm, 250 × 4.6 mm), mobile phase composed of methanol, acetonitrile, and monobasic potassium phosphate buffer (35:25:40, v/v), pH 3.9, and 0.05 M, with a flow rate of 1 mL/min, with detection at 284 nm, using HPLC. The method is linear in the concentration range 0.75 - 24 µg/ml, with precision and accuracy of the quality controls (0.9, 4, and 20 µg/ml) within 5% and 15%, for the analytical and bioanalytical methods, respectively, except for the lower limit of quantification (0.75 µg/ml) which is acceptable up to 20%. The quantification method proved to be selective, linear, precise, and accurate for measuring mefloquine in plasma and microdialysate samples from healthy and *P. berghei*-infected rat after oral administration of 55 mg of mefloquine.

Ethical approval

All procedures were reviewed and approved by the Committee on Ethics in the Use of Animals (CEUA) of School of Veterinary Medicine at the Federal University of Bahia (89/2019).

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Nano-architecture approaches to improve the antimalarial erythrocyte penetration: A study of POPPK/PD modeling in Plasmodium berghei infected rodents

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Our research group has been dedicated to improving the efficacy of antimalarial drugs by changing the nanocarrier architecture and have shown promising results, especially for quinine (QN) [1-3]. However, the studies are lacking to distinguish the biological effects of anionic (NC1) and cationic (NC2) coated nanocapsules containing QN. Our aim was to describe the PopPK/PD modeling of free QN, NC1, and NC2 in *Plasmodium berguei* infected rodents. From QN plasma profile and erythrocytes partition coefficient of QN, both in *P. berguei* infected rats, QN drug concentrations in whole blood were calculated and PopPK using Monolix-Suite™ 2020R1 (Simulation Plus, USA). Peter's suppressive test was assessed in mice [3] and then, interspecies translation were performed. A QN whole blood in mice [4] were modeled on Scientist and the hybrid constants were used to recalculated the populational parameters using the covariates from PopPK model. With this results, PopPKPD modeling were performed using Monolix. A two-compartment model with linear elimination described QN whole blood data in rats. NC1 and NC2 reduce CL and intercompartmental CL. NC2 reduced V2, as well as the weight influenced this parameter. A modified maximum inhibition model was able to describe the reduction of parasitemia in all treatments. NC1 and NC2 have been shown to decrease the IC50 by 100 and 500-fold, respectively. Nanoencapsulation of QN potentiated drug interaction with infected erythrocytes. In summary, results of PopPK and PopPK/PD indicated cationic nanocapsules are promising strategies to treat malaria.

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Is clindamycin a potential treatment for prostatitis?

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The presence of *Cutibacterium acnes* has been associated with chronic prostatitis, this situation has the potential to favor the appearance of prostatic tumors [1]. The effectiveness of clindamycin in the treatment of infections by this bacterium has been highlighted [2-4]. Male Wistar rats were divided into two groups (plasma, n = 8; prostate, n = 8) for pharmacokinetic studies. Clindamycin was administered at 80 mg/kg iv *bolus*. Carotid artery was cannulated for blood collection in the plasma group. For the prostate group, a microdialysis probe was implanted in the prostate gland. Collections were made over an 8-hour period. The pharmacokinetic parameters were determined by a non-compartmental approach (NCA). NCA resulted in the following values (plasma and prostate, respectively): elimination rate constant (h^{-1}): 0.33 ± 0.02 and 0.46 ± 0.07; half-life (h): 2.09 ± 0.15 and 1.55 ± 0.25 , peak concentration (mg/L) 35.82 ± 10.51 and 11.53 ± 3.24, area under the curve (h mg/L) 58.53 ± 14.78 and 19.40 ± 4.40, mean residence time (h): 2.51 ± 0.17 and 1.89 ± 0.17. Half-life values show that clindamycin is removed more quickly from the prostate than from the bloodstream. The concentrations in prostatic tissue were lower than the total plasma concentrations. In both cases clindamycin reached the minimum inhibitory concentration against *Cutibacterium acnes* [5]. Clindamycin could be suggested as an effective therapeutic option for studies on the treatment of chronic prostatitis.

Ethical approval

The experimental protocols involving animals were approved by the Ethics Committee on the Use of Animals of the University of Caxias do Sul (017/2018).

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Pharmacokinetics of a nanostructured cloxacillin formulation in goats naturally infected with *Corynebacterium pseudotuberculosis*

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The treatment of caseous lymphadenitis with antimicrobials is ineffective due the limited intracellular penetration [1]. However, the use of a nanostructured antibiotic may contribute to the effectiveness of therapy and microbiological cure, as demonstrated in studies with nanoencapsulated cloxacillin benzathine (CLOX-NS) against *Staphylococcus aureus* in bovine mastitis (patent WO/2011/150481) [1,2]. The aim was to construct a pharmacokinetic (PK) model of CLOX-NS in goats. Plasma PK data were obtained from an *in vivo* PK study previously performed in goats experimentally infected with *Corynebacterium pseudotuberculosis* [3]. With the concentration values over time a PK model was established in Monolix Suite 2020R1 software [4]. The PK model had an extravascular route of administration, with lag time, first order absorption, three compartments and linear elimination, and weight was considered a covariate. The estimated parameters were lag time (Tlag = 0.0018h), absorption constant (Ka = 1.19 h^{-1}), transit constants between compartments (K12 = 9.58 h^{-1}; K21 = 1.28 h^{-1}; K13 =9.77 h^{-1}; K31 = 0.8 h^{-1}), elimination constant (K = 104.9 h^{-1}) and central compartment volume (V = 2.61 mL). There was a correlation between weight and the parameters V (R= 0.94) and K (R= -0.53). The established PK model of CLOX-NC in goats is the first step for development of simulations to determine probability of target attainment (PTA) based on pharmacokinetic/pharmacodynamic integration.

Ethical approval

This study was approved by the Embrapa Institutional Animal Care and Use Committee under the protocol number 011/2014.

Financial support and acknowledgements

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K-PD models applications, limitations and perspectives: A systematic review

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Frequently on clinical trials and pre-clinical studies data on certain drug plasma concentration measurements can be sparse, unavailable or economically unfeasible, consequently PK/PD modeling can become non representative or require complex alternatives [1]. In this context K-PD models are useful, since they don't require PK data. Our aim is to assess the applications and limitations on the use of K-PD models on pre and clinical trials. To achieve it, the systematic review used "Cochrane handbook for Systematic reviews of interventions" as a guide and Rayyan app was used to conduce a double-blind review during selection phase. The research was conduced on PubMed, Cochrane Reviews, Web of science and Embase, using the keywords: "K-PD", "model", "clinical", "semi-mechanistic". In total 131 articles were found, only 37 were kept after all selection steps. The results showed the wide application K-PD models, varying from toxicity pre-clinical studies with animal envenomation [2] to a helpful decision tool on phase II and III clinical trials [3]. From all 37 articles only 9 were published by industry and 28 by universities and research centers. The main limitation detected was for non-linear elimination drugs, which can require more complex and adequate equations [4], in addition to more deep understanding PK and PD characteristics from the drug. Only 1 article with nonlinear elimination were found. In conclusion our findings asses that K-PD models are useful tools with wide application, however require robust knowledge to carry out the proper equations and drug biological characteristics to achieve a model clinically relevant.

Ethical approval

Do not apply.

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Pharmacokinetic evalution of free and nanoencapsulated Curcumin in rats

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Curcumin (CUR) is a natural compound with anti-inflammatory, antioxidant, antitumoral and antimicrobial activities [1]. CUR has low solubility and permeability, and nanotechnology is a promising alternative for improving delivery. Then, our research group nanoencapsulated CUR through functionalizing nanocapsules (NC) surface [2]. The aim of this work was to evaluate the PK of CUR nanoformulation after 2 mg/kg i.v. bolus administration in male Wistar rats (n=6/group). The groups evaluated were free CUR (F-CUR), NC1 (negative surface), and NC2 (positive surface). The quantification of CUR was performed by HPLC-PDA previously validated method. Non-compartmental analysis was evaluated by Pkanalix® from MonolixSuite™ 2020R1 (Simulation Plus, USA). Formulations presented nanometric size and zeta potential compatible with coating. At the same dose, $AUC_{0-\infty}$ was 49 ± 4, 276 ± 91 and 119 ± 56 μg.h.ml^{-1} for F-CUR, NC1 and NC2, respectively. Compared to F-CUR, NC2 reduced $t_{1/2}$, in view of Vd_{ss} and Cl_{Tot} was decreased in almost 7 and 2-fold, respectively. CUR from NC1 showed similar behavior, except in the Cl_{Tot}. The NC showed modified the PK of CUR.

Ethical approval

All experiments were performed after approval of the experimental protocol by the Ethical Committee for Animal Use of Federal University of Pampa (UNIPAMPA - Protocol CEUA 036/2018) and according to the guidelines of the Council for Control of Animal Experiments (CONCEA).

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Integration of PK/PD for dose optimization of cloxacillin sodium against Corynebacterium *pseudotuberculosis* for *in vivo* studies in goats

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The pharmacokinetic model allows for predicting the plasma concentration of a drug and thus estimating its therapeutic or toxic effect [1, 2]. We aimed to determine the first dose of intramuscular (IM) cloxacillin sodium (CLXS) against *Corynebacterium pseudotuberculosis* for *in vivo* study in goats. For this, a pharmacokinetic model with pharmacokinetic/pharmacodynamic (PK/PD) integration was constructed from data obtained from two plasma pharmacokinetic studies of CLXS, in goats (intravenous administration) and sheep (IM administration) [3-5]. After establishing the model, dose regimens were simulated to maintain the plasma concentration above the minimum inhibitory concentration (MIC = 4 μg/mL), for at least 40% of the time between doses interval [6]. The model was constructed using Lixoft® software, integrating the sheep's absorption constant (Ka) with the goat's volume of distribution (Vd) and clearance (Cl). The PK model was extravascular, with no delay, first-order absorption, one compartment, and linear elimination. The Ka (0.93 h^{-1}), Vd (0.32 L/kg), and Cl (0.28 kg/h) were determined, and then, two therapeutic regimens were simulated, one with intervals between administrations of 8 (q8h) and another of 12 hours (q12h), both with doses ranging from 10 to 200 mg/kg. According to the simulations, the doses of 10 mg/kg (q8h) and 30 mg/kg (q12h) reached concentrations higher than the MIC for 43 and 42% of the time between dose intervals, respectively. Through this study, it was possible to determine doses of CLXS with potential antibacterial effects to initiate *in vivo* studies.

Ethical approval

Since this is an *in silico* study, no animals were used.

Financial support and acknowledgements

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Populational pharmacokinetics modeling of free and nanoencapsulated curcumin in male wistar rats

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Curcumin (CUR) nanocapsules (NC) were developed by our research group and showed increase anti-inflammatory and neuroprotective effects [1-3]. Thus, the objective of this work was to be modeling PopPK from free (F-CUR) and nano-encapsulated CUR. NC1 (anionic NC) and NC2 (cationic NC) were prepared by nanoprecipitation method at 0,6 mg/ml concentration [1]. For the PK studies, healthy male Wistar rats were used. PK of free CUR (F-CUR), NC1 and NC2 were performed by administering 2 mg/kg i.v. bolus (n=6/group). Blood samples were collected and quantified in HPLC-PDA. A sequential modeling approach was used to describe CUR plasma concentration versus time profiles in rats using MonolixSuite™ 2020R1 (Simulation Plus, USA). For all groups structural model was a two-compartmental distribution and linear elimination. Besides that, model showed good precision of parameter estimates, with constant residual error and normal distribution. For NC1 and NC2 model, after fixed the F-CUR parameters, the kr (CUR release from NC constant) was added to model (derivate from CUR *in vitro* release test [1]). Then, F-CUR had similar V1 and V2 while V2NC1 and V2NC2 increased significantly. Between nanoformulations cationic NC2 increased 3 and 2-fold the values of V2 and Q, respectively. These results demonstrate the NC are promising as CUR drug delivery System.

Ethical approval

All experiments were performed after approval of the experimental protocol by the Ethical Committee for Animal Use of Federal University of Pampa (UNIPAMPA - Protocol CEUA 036/2018) and according to the guidelines of the Council for Control of Animal Experiments (CONCEA).

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Determination of a protocol for intramuscular tramadol with potential analgesic effect in zebrafish (*Danio rerio*)

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The zebrafish (*Danio rerio*) is widely used in studies of nociceptive stimuli, so is necessary to determine analgesic protocols in this species [1,2]. Human pharmacokinetic/pharmacodynamic (PK/PD) model allowed quantifying the analgesic effect after tramadol treatment [3]. For this reason, this study aimed to construct a PK/PD model to estimate tramadol doses by intramuscular route (IM) with potential analgesic effect in zebrafish. The model was constructed from the PK model of tramadol in zebrafish integrated with the PK/PD model in humans [3-5]. The established PK model was with extravascular administration, with no delay, first-order absorption, one-compartment distribution and linear elimination. The average values of the parameters absorption constant (Ka = 3.18 h^{-1}), central compartment volume (V = 0.067 mL) and elimination constant (K = 3.18 h^{-1}) were calculated. For the PK/PD integration, the parameters Hill coefficient, zero effect (E0 = 110.32 μA), maximum effect (Emax = 78.56 μA) and effective-concentration 50 (EC50 = 17.25 ng/mL) of the human tramadol model were used. IM protocols of tramadol were estimated to maintain the analgesic effect above 60% of the maximum effect throughout the interval between doses. Starting at the dose of 20 μg/animal, the maximum analgesic effect was achieved for 78 minutes, while doses of 30 and 40 μg/animal increased the duration of the effect by 12 and 24 minutes, respectively. Thereby, protocols starting at 20 μg/animal, every 78 minutes, are promising for the evaluation of the analgesic effects of tramadol *in vivo*.

Ethical approval

Since this is an *in silico* study, no animals were used.

Financial support and acknowledgements

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PBPK modeling as an alternative method of extrapolation interspecies that reduce the use of animals: A systematic review

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The use of animals in biomedical research is a practice that continues to prompt ethical and scientific debate due to animal suffering, reliability and limitations for extrapolation of data to humans [1, 2]. An alternative to traditional methods used for interspecies extrapolation is Physiologically Based Pharmacokinetic (PBPK), this approach simulates the anatomical structure of species using a computational model in which organs or tissues are represented as compartments interconnected by arterial and venous flows and are described by differential equations to predict the change in the concentration of the evaluated molecule [3]. The aim of this systematic review was to analyze published articles that used PBPK models for interspecies extrapolation in drug development and toxicological Assessment. For this, a systematic search was performed in PubMed, using the following search terms: "PBPK" and "Interspecies extrapolation", the revision was performed followed PRISMA guidelines. It was determined that the main source to obtain the anatomical and physiological parameters required for the development of the models were previous publications and rodents were the most used species; *in vivo* studies were mainly used for model validation. Additionally, 76% of the PBPK models were developed to extrapolate pharmacokinetic parameters to humans and the main application of the models was in health risk assessment. PBPK modeling is an alternative that allows the integration of *in vitro* and *in silico* data and parameters reported in the literature to predict the pharmacokinetics of chemical substances, reducing in large quantity the use of animals in research.

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Physiology-based pharmacokinetic modeling of Methylphenidate for the treatment of attention deficit Hyperactivity disorder in adults

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Physiology-Based Pharmacokinetic (PBPK) modeling is a mathematical approach that offers a unique modality to predict age-specific pharmacokinetics based on physiological parameters [1]. Attention Deficit Hyperactivity Disorder (ADHD) is a common neurodevelopmental disorder in childhood and may persist into adulthood, addressing approximately 2.5 to 5% of this group [2]. Methylphenidate (MPH) is a psychotropic stimulant drug and the first pharmacological choice for treating ADHD [3, 4]. But the various problems associated with its use require further investigation into its pharmacokinetics (PK) in treating ADHD, especially in human adults [5, 6]. A PBPK model for MPH was developed in the PK-SIM® software (version 8) utilizing *in silico*, *in vitro* drug metabolism, and *in vivo* pharmacokinetic parameters. Adult male and female individuals (18-65 years) were built considering CES1 as predominant metabolizing enzyme, half-life = 3.5 h, Cl = 10.5 L/h/kg, Vss = 2.23 L/kg. Regarding compound properties, MPH presents logP = 1.47, low protein binding = 15%, MW = 2.33.31 g/mol, experimental solubility = 1255 mg/L, pKa = 9.09 (strongest base) and no halogens in its structure. Only the oral extended-release (Concerta® 18 mg) formulation was considered for the model development. Once finished, the PBPK model will be beneficial in guiding optimal dosing regimens for MPH in the adult population. Financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES)-Finance Code 001.

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Progesterone intravaginal device pharmacokinetic model

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Progesterone-releasing intravaginal devices are used to control the cattle estrous cycle in the protocol of Fixed-time artificial insemination (FTAI), which is helpful for rationalizing livestock management [1]. The aim of this study is to create a pharmacokinetic model of progesterone-releasing intravaginal devices in cattle to support development of new protocols and intravaginal devices. The model used data from different intravaginal devices commercially available in Nellore (CIDR® 1,9g; Primer® 0,5g; Prociclar® 0,75g; Syncrogest® 1g) and Holstein animals (CIDR® 1,38g; PRID® 1,55g; Prociclar® 0,75g; Repro sync® 2g; Cue Mate® 1,56g; DIB® 0,5g; Syncrogest® 1g) [2]. It was developed with Lixoft® software, applying the constants calculated from Monolix suit in PK analix 2020R1 [3]. The PK model was extravascular, with delay, zero order, one compartment and Michaelis-Menten kinect. The values of the parameters were determined in absorption duration (Tk0) 11,84 $mg.h^{-1}$, lag time (Tlag) 2,78 h, Michaelis-Menten constant (Km) 4,09 $mg.L^{-1}$, maximum velocity (Vm) 11,87 $mg.h^{-1}$ and volume of the central compartment (V) 482,09 L^{-1}. Therefore, the model was able to estimate plasma concentrations of progesterone reliably with a homogeneous dispersion of the predicted value line, within the 90 % interval, even with a diverse data from two races with different devices and concentrations. From this work it is possible to establish new protocols and devices for FTAI.

Ethical approval

Since this is a silico study, no animals were used.

Financial support and acknowledgements

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Pharmacokinetic model of florfenicol in nile tilapias (*Oreochromis niloticus*) submitted to different rearing temperatures

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One of the most important factors for tilapia breeding is water temperature, since temperature variation influences the kinetics of antibiotics, therefore it is essential to optimize doses, as it may cause adverse effects and generate residues in animal products [1]. This study aimed to develop a pharmacokinetic (PK) model of florfenicol in Nile tilapia (*Oreochromis niloticus*) subjected to different rearing temperatures to estimate the plasma concentration of the products authorized in Brazil. For this purpose, a PK model was created from previously published data [2]. The plasma concentrations were estimated from treatments with doses of 10, 15 and 20 mg/Kg administered every 24 hours for 10 days, at temperatures of 18, 21, 26, and 30°C, using Monolix and Simulix Suite 2020R1 [3]. The parameters of lag time (R= -0,882), absorption (R=0,867), and clearance (R=0,807) were correlated with temperature. It can be observed that animals kept at 30°C have a median plasma concentration of half that of animals raised at 18°C treated with the same doses. This PK model serves as a basis for developing a pharmacokinetic/pharmacodynamic model for optimizing doses of florfenicol based on the rearing temperature of Nile tilapia.

Ethical approval

Since this is an *in silico* study, no animals were used.

Financial support and acknowledgements

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Association between plasma imatinib levels and response T_o the treatment of chronic myeloid leukemia in patients From Manaus, Brazil

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Imatinib mesylate (MI) is the drug of choice for the treatment of Chronic Myeloid Leukemia (CML). However, despite the majority of the results obtained with this therapy being positive, some patients still present a suboptimal therapeutic response or still develop some type of resistance [1]. Therefore, the aim of this study was to evaluate IM plasma levels in CML patients treated at a referral unit in Manaus and correlate them with variables that might interfere with these levels. Data from 52 patients were obtained through a standardized questionnaire containing clinical, sociodemographic,

lifestyle and use of other medication information, as well as an estimate of therapeutic adherence. Additionally, blood collection was performed to measure the plasma concentration of the drug using the HPLC-UV technique. Molecular studies were done to identify the presence of polymorphism in the ABCG2 C421A membrane transporter. Among the results obtained, most patients were male with a mean age of 52 ± 12.3 years (95% CI 49.0-55.9). There was a high variation in drug concentrations in the range from 0 to 4694 ng/mL, with a mean of 1558.59 ± 989.79 ng/mL (95% CI 1283.0-1834.1). Approximately two thirds of patients were classified in the drug level range considered therapeutic. Furthermore, there was a correlation between plasma concentration and higher molecular response. Additionally, most individuals had the normal genotype for the ABCG2 C421A polymorphism but further studies should be performed to reveal the role of this variable in the outcome of the disease in this population.

Ethical approval

The study was approved by the Research Ethics Committee at the Fundação Hospitalar de Hematologia e Hemoterapia do Amazonas, Manaus, AM, Brazil (approval number 2.540.371).

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PBPK model for predicting DDI between chloroquine and colchicine in healthy and infected patients

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New alternatives for the treatment of meloxicam should be explored due to resistance, such as drug repositioning [1] and encapsulation in nanoparticulate systems. How Chloroquine (CQ) [2] is a CYP inhibitor and Colchicine (CC) [3] is a CYP substrate, the study of synergism between the drugs for the treatment of malaria is important. As a pre-formulation study, the objective was to evaluate drug interactions (DDI) of CQ and CC for further co-encapsulation in nanocapsules. Physiologically based pharmacokinetic (PBPK) modeling was performed with the PK-Sim 9.0 software. The models for CQ and CC were independently optimized, using data in the literature [4,5,6] with oral administration (dose 300 mg CQ and 1 mg CC) in healthy and infected (altered hematocrit) patients. Subsequently, DDI was evaluated between CC and CQ. The models presented predicted error and mean fold error below 2%, demonstrating that the built model was able to describe the observed data. DDI of CQ and CC were analyzed, separately, with CQ inhibiting CYP3A4, CYP2D6, and P-gp to analyze if they may impact in PK of CC. There were no changes in CC AUC, Tmax, and Cmax for any assessment when CQ was inhibited. We also separately evaluated the impact on CQ PK when CC showed inhibition of CYP3A4 and P-gp, similarly, there were no changes in CQ AUC, Tmax, and Cmax. PBPK model was capable of characterizing the PK of CQ and CC in healthy patients and sick patients and demonstrated that there was no drug interaction under the conditions tested when administered, simultaneously.

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Elevated vancomycin through levels in patients admitted to an university hospital from Manaus, Brazil

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Vancomycin is a key antibiotic in the therapy of Gram-positive infections caused mainly by *Staphylococcus* sp. Several studies have reported that inadequate dosage is associated with therapeutic failure, bacterial resistance and toxicity [1]. Therefore, therapeutic drug monitoring (TDM) of this agent is essential to ensure the achievement of clinical results and minimize adverse effects according to the literature. Thus, this study aimed to evaluate the through serum levels of vancomycin in patients admitted to an university hospital. An analytical-observational cross-sectional study was carried out from January to October 2020 to attain this goal. Medical records including sociodemographic, clinical and laboratorial data were collected and the plasma levels of vancomycin were achieved using a previously validated HPLC-UV method [2]. In total, 50 patients were included in this investigation. Most patients were male (60%) and the mean age observed was 50.76 ± 19.47 years. The mean through vancomycin concentration was 40.34 ± 24.21 mg/L. Furthermore, approximately half of the patients had serum levels considered to be toxic (> 30 mg/L). The association of higher levels in patients on polypharmacy regimen and with acute renal failure was also observed. The results obtained here call attention to the importance of TDM in order to adjust drug therapy of vancomycin.

Ethical approval

The study was approved by the Research Ethics Committee at the Universidade Federal do Amazonas, Manaus, AM, Brazil (approval number 3.858.967).

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Therapeutic drug monitoring of phenobarbital in saliva in epileptic dogs

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In veterinary medicine, therapeutic monitoring of phenobarbital (PB) in patients under antiepileptic treatment is performed routinely in blood samples. This method is invasive and, in some patients, impractical due to the difficulties involved in its handling. In this work, we investigated the usefulness of saliva as monitoring fluid. Fourteen epileptic dogs under treatment with PB were included. Serum and saliva samples were extracted simultaneously at steady state and concentration were analyzed using Monolix® 2021R1 software (Lixoft SAS, Antony, France). Saliva/serum fraction (f), apparent clearance (CL/F) and covariate analysis were performed. A monocompartmental disposition model was assumed and typical estimates for absorption rate constant (ka) and apparent volume of distribution (Vd/F) fixed based on bibliographic information [1,2]. A positive correlation between PB concentrations in saliva and serum was found. A monocompartmental model with autoinductive elimination CL was found to better describe PB data [3,4]. Seizure control and age were found to significantly affect f, while CL was significantly affected by WT and age.

Equation best describes CL and f were:

CL=CLpop(WT/20)^3/4x(AGE/5)^(β_AGE).

f = fpopx(AGE/5)^βf_AGExe^β_f_CONTROL_2 (if CONTROL=2)

Being CONTROL=2 no responsive patients, 20 the average WT in kg, and 5 the average age in years of the studied population

Discussion and Conclusion

There is a good correlation between saliva and serum concentrations of PB being saliva a suitable fluid for therapeutic monitoring of PB in dogs. The difference in f between responders and refractory patients could be due to an overexpression of efflux transporters in the salivary gland of unresponsive patients.

Ethical approval

This research was approved by the Committee of Ethics in the Use of Animals (CEUA-FVET) of the School of Veterinary Medicine-Protocol number 799

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Use of PK/PD modeling to minimize the environmental impact of the use of doramectin 3.5% against *Rhipicephalus Microplus* in cattle

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The use of antiparasitic drugs contributes significantly to the increase of productivity in cattle breeding, and optimization of the dosing regimen is essential for greater efficacy and reducing residue elimination in the environment [1]. The aim of this study was to develop a pharmacokinetic/pharmacodynamic (PK/PD) model of 3.5 % doramectin protocols against *Rhipicephalus microplus* in cattle. For this purpose, previously published PK and PD data were used [2]. Plasma concentrations and efficacy against ticks were estimated from single dose (700 µg/Kg on day 0), two-dose (350 µg/Kg on days 0 and 14) and four-dose (175 µg/Kg on days 0, 14, 28 and 42) regimens, using Monolix and Simulix Suite 2020R1[3]. The PK model had extravascular route of administration, lag time, first-order absorption, one-compartment distribution and linear elimination; the PD model had direct response, Emax for drug action, baseline = 0 and sigmoidicity. Means of estimated parameters were: lag time (Tlag = 2.49 d), absorption rate constant (Ka = 0.089 d-1), clearance (Cl = 356 mL d-1), volume of the central compartment (V =2036.8 mL), maximum effect (Emax = 100 %), concentration producing 50 % of Emax (EC50 = 4.88 µg/mL) and Hill's coefficient (gamma = 1.29). The model was able to estimate a protocol that increases the residual effectiveness period by 75 % without increasing the environmental impact.

Ethical approval

No animals were used in this *in silico* study.

Financial support and acknowledgements

The study received financial support from the Coordination for the Improvement of Higher Education Personnel (CAPES), National Council for Scientific and Technological Development (CNPq - 448011/2014-0), Brazilian Agricultural Research Corporation (Embrapa) and Undergraduate Scholarship Research Program from Federal University of Lavras (UFLA).

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Development of drug-drug interaction models *in vitro* for macrocyclic lactones and their understanding

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Combination anthelmintic formulations have gained traction in the role of drugs in the control of parasitic nematode in recent years. While the use of pharmacometrics modeling is well-established

in the drug formulations development process, previously there is an increasing need for a better quantitative biological understanding of the drug-drug interaction (pharmacodynamic). In this study, we assessed the synergism of two anthelmintic drugs, eprinomectin (EPM) and ivermectin (IVM), widely used in veterinary medicine. In the animal model *Caenorhabditis elegans* [1], we used an infrared motility assay to measure EPM and IVM effects on worm movement over time. Different EPM/IVM concentration ratios were used and synergy scores determined with two different methodology to determine the drug exposure (AUC vs. Count at last time). The study discussing four modeling efforts in the field, applying highest Single Agent (HSA), Loewe additivity model (Loewe), Bliss model (BLISS) and zero Interaction Potency (ZIP) models [2]. The results the Synergy Score values were lower than ± 0.5 for all models, clearly indicating that there is no synergy between both macrocyclic lactones. This study show that it is more relevant to prioritize the exposure time of each individual drug than to combine them to improve their effects. The results highlight the utility of *C. elegans* to address preliminary pharmacodynamics studies, particularly for drug-drug interactions. Models *in vitro* can be further integrated to facilitate preclinical to clinical translation and help researchers better understand the mechanisms of drug-drug interactions to help achieve a more favorable therapeutic.

Ethical approval

The study does not require approval.

Financial support and acknowledgements

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Simulation of variable rate infusion regimens for propofol in dogs

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Variable rate infusion (RCI) aims to adapt the infusion rate for constant plasma concentration over time. Pharmacokinetic (PK) models coupled with RCI systems has been increasingly used in veterinary medicine [1]. The objective of this study was to develop a PK model of propofol for simulations of different regimens of bolus induction associated with RCI in dogs. Raw data of propofol plasma concentration over time in dogs was used for the development of the PK model [2]. Induction and maintenance bolus protocols with RCI were simulated in order to maintaining plasma concentration within an established target for magnetic resonance imaging (3 - 6 ug/mL) and dental procedures (2.5 - 4.7 ug/mL). Monolix and Simulix Suite 2020R1 was used for modeling and simulation [3]. The established PK model had no delay, three-compartment distribution and linear elimination. Protocols able to keep consistent plasma concentration over time had 2, 3 and 4.5 mg/Kg bolus induction, and initial rates of 200, 300 and 450 ug/Kg/min, with rate reductions of 10, 15 and 25 ug/Kg/min every 20 minutes, respectively. For MRI, 3 - 300 - 15 protocol kept the mean plasma concentration within the target, as well as the 2 - 200 - 10 protocol demonstrated data within the target for dental procedures. The PK model was able to estimate plasma concentration of propofol in dogs, and enabled the investigation of rate adjustments to maintain propofol plasma concentration within the established range.

Ethical approval

Since this is an *in silico* study, no animals were used.

Financial support and acknowledgements

This study was funded by the Coordination for the Improvement of Higher Education Personnel (CAPES), the National Council for Scientific and Technological Development (CNPq), the Foundation for Research Support of the State of Minas Gerais (FAPEMIG), and the Undergraduate Research Scholarship Program of UFLA.

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Modeling & simulation of tramadol in red-eared turtle (*Trachemys scripta scripta*)

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In reptiles and avians, the administration site can impact the pharmacokinetic profile and the therapeutic effect due to renal and/or hepatic portal deviation [1,2]. Thus, modeling & simulation (M&S) helps to predict the pharmacokinetics and effect in these animals. The objective was to build a pharmacokinetic/pharmacodynamic (PK/PD) model to simulate therapeutic protocols of tramadol, after administration on the forelimb and hindlimb, in the *T. scripta scripta* species. The data of plasma

concentration and antinociceptive effect were obtained from literature, from which it was constructed a PK/PD model, and protocols were simulated at a dose of 10mg/kg at different intervals of administration [12 (q12h), 24 (q24h) and 48 (q48h) hours] and administration routes (hindlimb or forelimb) [2]. For M&S, the Lixoft® 2021R1 package was used [3]. The PK model was of extravascular administration, without delay, with first-order absorption, three compartments and linear elimination, while the PD model was with a turnover response, stimulation-producing action and with sigmoidity. The protocol of 10mg/kg (q12h) maintained the antinociceptive effect for a longer time, in both administration routes. The forelimb had a higher plasma concentration, but the

hindlimb had a greater effect, which may be related to the hepatic portal shunt, which results in the metabolization of tramadol into O-demethyltramadol hydrochloride, its active metabolite. Because of the M&S, it was possible to perceive the effect of the hepatic port system on the metabolization of tramadol, and consequently, the difference in the effect depending on the route of administration [4]

Ethical approval

Since this is an *in silico* study, no animals were used.

Financial support and acknowledgements

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In vitro release and population pharmacokinetic modeling of free and nanoencapsulated meloxicam in male Wistar rats

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Computational modeling through simulations proves to be a promising alternative in the drug research, development and innovation (RD&I) phases. The aim of this study was to characterize a pharmacokinetic model (PK) in male *Wistar* rats (n=10/group) treated with free meloxicam (F-MLX) and encapsulated in cationic nanocapsules (NCM). The pharmacokinetics of F-MLX and NCM were performed at a dose of 5 mg/kg iv and plasma concentrations were quantified on HPLC-PDA. Concentration versus time profiles were modeled using a population pharmacokinetic (popPK) approach in MonolixSuite™ 2020R1 (Simulation Plus, USA). *In vitro* release experiments using the dialysis bag method in pH 7.4 phosphate buffer medium were performed, aiming to describing release of MLX in the blood. Based on this, the release constant (kr) was calculated (DDSolver) and added to the NCM model. A two-compartment model with linear elimination, combined1error and normal distribution described the F-MLX. The values obtained were fixed to NCM modeling, together with the kr value previously calculated. The model that best described it was the three-compartment model with linear elimination, combined1 error and normal distribution. The F-MLX and the NCM had a similar Volume of Distribution (V1=0.028, V1NC=0.03; V2=0.027 and V2NC=0.033 mL, respectively). However, for NCM, there was a third compartment, with a relatively small intercompartmental clearance (Q3NC =0.0095 L/h), and a relatively large V3NC (0.21 mL), when compared to the other compartments. These results demonstrate that it was possible to describe the behavior of nanoencapsulated MLX using *in vitro* and *in vivo* data.

Ethical approval

Protocol was approved by the Ethical Committee for Animal Use of UNIPAMPA (Protocol Nº030/2021).

Financial support and acknowledgements

This study was financed by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES), Rio Grande do Sul Science Foundation (FAPERGS), grants #88881.506652/2020-01 and #19/2551-0001970-0, National Counsel of Technological and Scientific Development (CNPq) grant #309401/2020-8. We want to thank Simulation Plus for MonolixSuite™ license.

Development and validation of a microsampling strategy to optimize antituberculosis therapy

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Tuberculosis (TB) is caused by the *Mycobacterium tuberculosis* complex and the Directly Observed Therapy Short-course (DOTS), recommended by World Health Organization, includes first-line anti-tuberculosis drugs such as isoniazid (INH), rifampicin (RFM), pyrazinamide (PZA) and ethambutol (ETB) [1,2]. At present, the standard method of blood sampling for therapeutic drug monitoring (TDM) is venipuncture; unfortunately, it can cause discomfort in TB patients. Dried blood spots (DBS) are a minimally invasive method for the collection of whole blood [3]. The aim of this study was to develop a UPLC-MS/MS method for quantification of anti-tuberculosis drugs in plasma and DBS to demonstrate its clinical application in TDM. Separation and detection conditions were optimized to quantify the four anti-tuberculosis drugs in both samples. For plasma, the method was linear from 0.5-25 μg/mL for RFM, 0.5-12 μg/mL for ETB, 0.5-20 μg/mL for INH and 5-120 μg/mL for PZA. For DBS, the method was linear from 0.375-24 μg/mL for RFM, INH and ETB and 2.031-130 μg/mL for PZA. The methods developed were proved to be precise (inter- and intra-assay), selective and accurate; matrix effect was demonstrated to be within the established limits. Short- and long-term stability, as well as freeze-thaw cycles for plasma and DBS were determined. The method was applied in the monitoring of the anti-TB drugs in 9 TB patients. Finally, anti-TB drugs plasma concentrations were related to DBS concentrations by linear regression to determine the concordance between both measures. The plasma results are clinically useful for decision-making in dose adjustments.

Ethical approval

The present work was approved by "Comité de ética en investigación del Hospital Central Dr. Ignacio Morones Prieto" with registration 67-21 and "Comité de ética en Investigación y Docencia de la Facultad de Ciencias Químicas (CEID-FCQ)" with code CEID2021-012-S.

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A model for the changes in the pharmacokinetics of Quercetin-3-o-rutinoside in an extract of calyces from *Physalis peruviana* in rats

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Quercetin-*3-O*-rutinoside (rutin) is the marker compound of a hydroethanolic extract of calyces from *P. peruviana* L. with hypoglycemic activity [1]. The pure flavonoid belongs to class IV of the biopharmaceutical classification system, but the complex mixture of compounds in the extract increases its solubility [2] and produces pharmacokinetics alterations in animals [3]. The aim of this work was to develop a population pharmacokinetic model (popPK) describing the changes in the pharmacokinetics of rutin due to the extract in rats. Blood plasma concentrations of rutin and quercetin (representing rutin metabolites) were obtained from rats administrated with at least two independent doses of pure rutin or extract intravenously and orally (n = 5), using a validated bioanalytical method. The popPK modeling was carried out in Monolix 2019R1. Blood plasma concentration-time profiles of i.v. rutin were fitted to a model of two compartments with linear elimination. Only quercetin was detected from p.o experiments, and their concentrations were fitted to a model with double absorption. The analysis of covariates showed that the extract increases the volume of distribution and the elimination rate of rutin, but also increases the absorption rate and decreases the time for the second absorption of their metabolites, in a dose-dependent manner. These observations were consistent with the non-compartmental and statistical analysis of the data. The popPK modeling approach let to better describe the complex pharmacokinetics of rutin and their metabolites and the variability due to the extract dose. The models could be used in new researches of the group.

Ethical approval

The study was approved by the ethics committee of the science faculty (Act 06, 2015, project 40831, 22 June 2015).

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Effects of sex on pharmacokinetic of meloxicam in rats: A POPPK study

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The pharmacological response may vary in men and women due to their anatomical, physiological and hormonal differences. Studies related to the influence of gender on the pharmacokinetics (PK) of drugs allow us to understand this variability in the pharmacotherapeutic response [1]. MLX, a non-steroidal anti-inflammatory drug used to treat pain, inflammation, and rheumatoid arthritis, has been widely used by both sexes [2]. In studies with male and female rats, there are several discussions about PK and the influence on sex is still controversial for MLX [3]. The aim of this study was to evaluate the influence of sex on the PK of MLX after 5 mg/kg, iv dosing in male and female rats. Plasma data from the male (n=11) and female (n=12) rats amounting to 319 observations were analyzed using the MonolixSuite™ 2020R1 (Simulation Plus, USA). The best-fit structural model is the two-compartment model with linear elimination. Inter-individual variability and correlation between Clearance (Cl) and Volume of distribution of the central compartment (V1) were entered. In covariate analysis, the female gender in Intercompartmental Clearance (Q) was statistically significant. The values for the fixed effects agree, as well as the coefficient of variation for all parameters and the visual predictive check with the observed data. Then, the final PopPK model was able to describe MLX PK for both sexes and demonstrated that females impact the drug plasma clearance.

Ethical approval

The experimental protocol was approved by the Ethics Committee on the Use of Animals of the UNIPAMPA (030/2021).

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Physiologically-based pharmacokinetic modeling of a novel tizanidine hydrochloride syrup for pediatric use

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Tizanidine hydrochloride (TZ) is a muscle relaxant and is an effective and safe drug for decreasing spasticity associated with spastic cerebral palsy in children. The problem is that TZ is sold only in tablets[1]. To bypass it, a syrup containing TZ for children was developed[2]. The aim of this work was to predict TZ plasma concentrations in children through a physiologically-based pharmacokinetic (PBPK) model after the administration of this novel TZ syrup. Plasma drug concentration data in adults after oral administration of TZ tablets (4mg and 8mg) were collected and used to build the PBPK model. After syrup formulation inclusion, the model was scaled to children accounting for age-related changes. Thus, TZ plasma concentrations were predicted through the model in children aged from 0 to 2 years and from 2 to 12 years. Pharmacokinetics (PK) parameters were evaluated. The model predicted TZ plasma concentration in adults was well performed and the average fold error (AFE) fits the 2-fold acceptance criteria in both scenarios (1.79 and 1.19). Eldest children's clearance (CL) and volume of distribution (Vd) were 2.65 ± 0.48 L/h/kg and 7.22 ± 0.74 L/kg, respectively. For the youngest, estimated values were 2.33 ± 0.74 L/h/kg and 6.64 ± 1.13 L/kg. Although, the area under the curve (AUC) for this group (6157.21 ± 12097.82 ng.min/mL) shows more variability in comparison to the eldest (2029.30 ± 707.71 ng.min/mL). In conclusion, TZ syrup seems to be safe for eldest children. On the other hand, more studies are necessary for the youngest population.

Ethical approval

Not applicable.

Financial support and acknowledgements

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Pharmacokinetics of methadone in adults patients undergoing cardiac surgery with extracorporeal circulation

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Methadone has characteristics that make it attractive for postoperative pain management after cardiac surgery. The use of cardiopulmonary bypass (CPB) involves changes that can alter pharmacokinetics and clinical response to drugs [1]. The aim of this work was to describe the pharmacokinetics of an intravenous bolus dose of methadone in adult patients undergoing cardiac surgery with CPB. We prospectively studied 18 patients between 45 and 75 years scheduled for cardiac surgery with CPB. A single bolus of 0.2 mg/kg methadone was administered in anesthetic induction. Then arterial blood samples were taken during 24 h from each patient. Methadone concentrations plasma analysis was performed using a spectrofluorometric method. Population pharmacokinetic analysis was performed with the program NONMEM. One-and two-compartmental models were used to describe the time profile of methadone plasma concentrations. All 18 patients completed the study. A two-compartment structural model produced a better fit of the data than a one-compartment model. (Decrease in the OFV of 297 points, $p<0.01$) The influence of CBP on methadone PK was better described by an increment in elimination clearance during CBP. Incorporation of this new parameter greatly improved the fit of the model with a decrease in OFV of 43 points. ($p<0.01$). The use of CPB alters the pharmacokinetics of methadone. These changes were adequately characterized by an increase in elimination clearance during CPB using a 2-compartment model with linear elimination. The clinical impact of the observed CPB changes in methadone pharmacokinetics are probably negligible with current dosing scheme.

Ethical approval

The study was registered at clinicaltrials.gov NCT05075265.

We obtained Clinical Ethics Committee approval and patient informed consent before starting the study. ID protocol 200323005.

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Development and validation of a physiologically based biopharmaceutics modeling to assess the bioequivalence of furosemide oral products in dogs

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Furosemide is widely used in cardiological therapies in canines [1], however there is a lack of knowledge regarding the dose-exposure-response relationship and the impact of multi-source drug products with no bioequivalence evaluation. In this work, we aimed to predict the relative bioavailability between two furosemide oral local products available for use in dogs by means of physiologically based biopharmaceutics modeling (PBBM), integrating formulation-related parameters estimated through *in vitro* dissolution testing in a PBPK model [2,3]. Further, the model predictions were validated with a bioequivalence study in healthy dogs (n=6). A previously reported furosemide PBPK model for humans [4] was adapted to dogs in PK-Sim® and MoBi® [5]using literature pharmacokinetic data in plasma and urine after intravenous and oral administration [6,7]. The model was optimized including a concentration-dependent increase in renal blood flow, affecting furosemide renal excretion. Dissolution profiles from two local products obtained in biorelevant conditions were fitted with a gompertz model and included in the PBPK model to describe drug dissolution. Single-dose furosemide Cmax and AUC for both formulations were predicted with a relative bias below 30%. The predicted Test/Reference GMR in dogs (n=1000) were 0.156 for $AUC_{0\text{-}inf}$ (90%CI 0.102-0.249) and 0.146 for Cmax (90%CI 0.105-0.202), while the observed results were 0.186 for $AUC_{0\text{-}inf}$ (90%CI 0.132-0.260) and 0.203 for Cmax (90%CI 0.129-0.320). The PBBM approach predicted adequately furosemide pharmacokinetics and the relative product performance in dogs.

Ethical approval

The protocol for the *in vivo* determination of plasma concentrations of furosemide in dogs was approved by the Ethics Commission for the Use of Animals (CEUA) of the Honorary Commission for Animal Experimentation (CHEA) under protocol number 1174 of the period 2021-2022.

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Population pharmacokinetics of moxifloxacin in patients from a tertiary care center in Mumbai, India

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Moxifloxacin (MFX) is one of the drugs recommended by the World Health Organization to be included in the regimen to treat multi-drug resistance tuberculosis (MDR-TB) and India is the country with the highest absolute burden with 27% [1]. The aim of the study was to describe the pharmacokinetics of MFX in an Indian population with MDR-TB. A total of 234 concentrations from 39 MDR-TB Indian patients were analyzed. Twenty four patients were female, with median weight, fat-free mass, and age of 56 (range 35–103) kg, 40 (26–73) kg, and 28 (17–46) years, respectively. The pharmacokinetics of MFX was best described by a two-compartment model with first-order elimination and transit compartment absorption. The allometry was implemented using fat-free mass and weight. The typical values for clearance, intercompartmental clearance, central and peripheral volume were 10.6 L/h, 4.45 L/h, 90.7 L, and 50.8 L, respectively. Between subjects variability on clearance was 31.2% described by an exponential function. The unexplained variability was 3.96% (heteroscedastic component) and 0.16 mg/L (homoscedastic component), additionally an extra additive error was estimated for unobserved data in 0.35 mg/L. The simulations showed that a MFX dose of 800 mg/24h is adequate if the minimum inhibitory concentration (MIC) is 0.5 mg/L. However, for a MIC of 0.25 mg/L a dose of 400 mg/24h is enough to achieve the therapeutical target of area under the curve of free MFX/MIC > 53[2] in more than 90% of the simulated patients.

Ethical approval

Institutional Review Boards at the PD Hinduja National Hospital and Medical Research Centre (884-14-ZFU) & Johns Hopkins University School of Medicine (IRB00076738).

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Implementation of a pilot therapeutic drug monitoring program for antibiotics in pediatric patients

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Antibiotics are among the most utilized drugs in pediatrics. Nonetheless, there is a lack in pharmacokinetics (PK) information for this population, and dosing criteria may vary between healthcare centers [1]. Physiological variability associated with maturation in pediatrics makes it difficult to reach a consensus on adequate dosing, and it is accentuated in more vulnerable groups, such as critically ill or oncology patients [2]. Therapeutic drug monitoring (TDM) is a useful practice that allows dose optimization and attainment of antibiotic-specific PK/pharmacodynamic (PD) targets [3]. The aim of this study was to implement a TDM program for antibiotics in pediatric patients on a local hospital in which this service is not available. A liquid chromatography coupled to tandem mass spectrometry method for the quantification of clindamycin, fluconazole, linezolid, meropenem, metronidazole, piperacillin and vancomycin in plasma was developed and validated based on FDA guidelines. A total of 23 pediatric patients aged 2-16 years were monitored either with an optimized sampling scheme or through opportunistic sampling. PK parameters were estimated through a Bayesian approach using the Abbottbase PKS software. The most frequent diagnostic was neutropenic fever and pneumonia in immunocompromised oncology patients (49%), followed by others such as appendicitis (17%); 43 TDM events were registered and 27 (63%) of them required dose adjustments as follows: 14 patients were underdosed, 4 were overdosed and 9 patients needed infusion rate adjustments. These results show high variability in target attainment throughout pediatric patients with standard dosing regimens, showcasing the need for TDM in especially vulnerable subpopulations.

Ethical approval

The study was submitted and approved by the Hospital's Research and Ethics Committee (Registration number: 69-21).

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External evaluation of population pharmacokinetic models for tacrolimus in Uruguayan kidney transplant patients

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Tacrolimus (FK) is a calcineurin inhibitor commonly used as part of immunosuppressive therapy in renal transplant patients to prevent graft rejection. Due to its high intra- and inter-individual variability as well as its narrow therapeutic index, FK therapeutic drug monitoring (TDM) is indispensable. In this context, model-informed precision dosing (MIPD) can lead to safer and more effective treatments [1], and several population pharmacokinetic (popPk) models have been reported for FK and are available for prospective implementation. However, the large variability among these models in the predicted exposure of typical patients prevents its application with no previous validation. The aim of this work was to conduct an external evaluation of popPk models of FK in kidney transplanted adults using retrospectively collected TDM data from 39 hospitalized patients (998 observations) to assess the *a priori* and *a posteriori* predictive performance using the covariate information and Bayesian forecasting, respectively. A mean relative bias (rBias) between +/- 20% with the 95%CI including zero was considered clinically acceptable. The relative root mean square error (rRMSE) [2], was computed to assess precision. Other metrics for accuracy and precision were also considered. A total of 28 published models were evaluated showing significant variability in the predictive performance. Bayesian forecasting showed to significantly improve the model's accuracy and precision. Nine models [3-11], proved to meet the requirements to be considered suitable for potential dosages adjusts of FK in our population. Those models included CYP3A5 polymorphism, hematocrit, post-operative-day, and prednisone dose as relevant covariates.

Ethical approval

This work is part of the Project "Desarrollo de Modelos predictivos y farmacogenéticos en pacientes trasplantados renales en tratamiento con Tacrolimus" which was evaluated and accepted by the Ethics Committee of the Hospital de Clínicas Dr Manuel Quintela (Approved 03/2021).

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Physiologically based biopharmaceutics modeling to predict bioequivalence results: A proof-of-concept study

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Omeprazole is a widely used proton pump inhibitor commercialized as capsules containing enteric-coated microgranules due to its liability at acidic conditions. The drug-induced increase in gastric pH can affect the enteric-coating performance under multiple-dosing, affecting the oral bioavailability. The aim of this work was to evaluate a physiologically based biopharmaceutic modeling (PBBM [1]) approach to predict the relative bioavailability achieved by omeprazole multi-source products after single and multiple doses. *In vitro* dissolution testing was performed in I-USP apparatus including an acidic stage followed by a neutral stage. Acidic stages with pH 1.2 and 4.5 were used to simulate the gastric pH after single and multiple doses, respectively. Resistance of the enteric coating at increasing pH (3 to 5) was tested in IV-USP apparatus. Omeprazole pH-dependent degradation kinetics were also characterized *in vitro*. This information was quantitatively included in a semi-PBPK omeprazole model, developed using Simulx® [2] by adapting and extending a previously published model [3]. Significant product-related differences in dissolution rates and enteric-coating performance were found. Relative bioavailability after single and multiple oral doses for multi-source products was predicted and validated in a bioequivalence study performed between the most different products in healthy subjects (n=12). Interestingly, the model predictions were found to accurately describe observations in men, since we observed a sex-by-formulation interaction in the bioequivalence outcome. The semi-PBPK model was further refined to characterize the physiologically based mechanism behind this interaction, finally achieving accurate predictions for both sexes. This work stands as a proof-of-concept for the use of PBBM in bioequivalence applications.

Ethical approval

The bioequivalence clinical trial was approved by the ethics committee of Faculty of Chemistry of the Universidad de la República, Uruguay.

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In silico pharmacokinetic predictions for the design of a Novel gastroretentive release system for sildenafil citrate

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In silico simulations have been suggested to reduce time and effort in the pharmaceutical development of drug products [1]. Currently, this approach has been successfully predicting *in vivo* performance of drugs and could be useful in the design of novel modified-release dosage forms (MR)[2]. Gastroretentive drug delivery systems (GRDS) are MR designed to prolong their residence time in the upper gastrointestinal tract [3-4]. Thus, this work proposes a novel GRDS for sildenafil citrate and evaluates the proposed formulations by *in silico* pharmacokinetic (PK) modeling and simulations with GastroPlusTM. A compartmental model with mechanistic absorption was built. The predictive power for the pharmacokinetic parameters of C_{max}, T_{max}, and AUC was proven. An *in silico* simulation study of a single dose of 60 mg in five formulations (F1-F5) was carried out and compared with the adopted target profile, the administration of 20 mg of immediate-release (IR) three times a day. It was possible to verify that formulations F2 and F4, which have a higher dissolution rate than the others, present $AUC_{0\text{-}inf}$ and $AUC_{0\text{-}t}$ very close to 100% in this comparison with the IR target profile. However, the absorption rate measured by the C_{max} parameter was below 80% for formulations F2, F3, and F5, indicating that the delay in drug release by the formulation also leads to a delay in the absorption process. Thus, it is concluded that gastroretentive systems are a promising alternative for controlling the dose release of drugs such as sildenafil, which have a narrow absorption window due to their solubility characteristics.

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Clozapine-loaded nanocapsules improve antipsychotic activity in rats: Building a PK/PD model to discriminate nanocarriers in the development step

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Here, we used a popPKPD modeling to discriminate the pharmacokinetics and pharmacodynamics of different coated-nanocapsules (NCs) containing clozapine (CZP), an antipsychotic with low solubility and serious adverse effects. A sequential model building approach (MonolixSuite™-2020R1-Simulation Plus-USA) was conducted integrating *in vitro* CZP release, plasma profiles after administration of different NCs (NC1, NC2, and NC3), and percentage of head movements in stereotyped model induced by amphetamine in rats [1]. A two-compartment model with linear elimination described CZP plasma data after i.v. administration of CZP solution. These parameters were fixed to subsequently analyze CZP plasma data for NCs. Two additional compartments were inserted to describe NC1 and NC2 distribution (anionic surface), while for NC3 (cationic surface) was included a third compartment. For all NCs, the rate constant for CZP release (*kr*) was calculated from *in vitro* data using a first-order model with Fmax. We found that nanoencapsulation promotes an increase in the Vd for all groups, particularly NC3. An indirect turnover model for drug inhibition was found to characterize the PK/PD of CZP. In this analysis, one important limitation was the absence of PK data after intraperitoneal administration. Under the assumption that this route provides complete CZP bioavailability, results show a formulation-dependent plasma IC50, with 20-, 100-,

and 700-fold reduction relative to CZP solution (NC1, NC2, and NC3, respectively). This indicates that the dissimilar distribution achieved by NCs can alter the plasma exposure-response relation for CZP. The proposed popPK/PD model proved to be a useful tool for nanoformulation development.

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Approximation to the population pharmacokinetic model of a self-emulsifying delivery system of an extract from *Physalis peruviana*

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The hydroalcoholic extract of *Physalis peruviana* presents poor oral bioavailability since it has low solubility and permeability according to previous investigations. A self-emulsifying delivery system (SEDDs) was formulated in order to improve the bioavailability of the extract, mainly the flavonoid rutin, its major therapeutic compound [1, 2]. The objective of this work is to perform the population pharmacokinetic modeling of the extract formulated with the SEDDs that describes the pharmacokinetic behavior of the extract in Wistar rats. The modeling was performed by the software Monolix 2021R1 (Lixoft®, Paris, France) and the model was chosen based on the observation of graphs, Akaike information criteria (AIC) values and accuracy of the estimated parameters. The final PopPK model has dual compartment, double absorption order 1 and order 0, a lag time for the second absorption and Michaelis Menten elimination [3]. Besides to the high bioavailability of the extract formulated in the SEDDS, the main difference with the unformulated extract is the Michaelis Menten elimination. That results could be explained since the high plasma levels of rutin (from extracts) after oral administrations SEDDs, cause changes in metabolic processes rate such as transport to the intestinal lumen and active tubular secretion can be saturated [4-6].

Ethical approval

The study was approved by the Ethics Committee of the Science Faculty of Universidad Nacional de Colombia (Act 06, 2015, project 40831, 22 June 2015).

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Physiologically based biopharmaceutics model applications to ANVISA: A brief characterization can evidence some regulatory trends and needs

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Physiologically based biopharmaceutics modeling (PBBM) applications are increasing for all regulatory agencies [1]. PBBM has been used by pharmaceutical companies in distinct scenarios as a guide for drug development, ensuring quality and reducing clinical trials, and as support for regulatory decisions [2]. The aim of this work was to investigate and characterize the PBBM-related applications to ANVISA, from 2020 to 2021, considering two offices (CETER and GQMED). The inclusion criteria were applications including PBBM analysis to support chemistry, manufacturing, and controls (CMC) posta-pproval changes and bioequivalence assessment. Applications including physiologically based pharmacokinetic modelling (PBPK), population pharmacokinetic modeling (PopPK), and modeling and simulation (M&S) for other purposes were not considered. The results reveal a total of 4 applications, 2 per year, which encompass drugs classified according to Biopharmaceutic Classification System (BCS) as class I/III (n=2), class II (n=1), and class IV (n=1). All applications aimed to support bioequivalence assessment, half of them requesting biowaiver for register of lower (n=1) or higher (n=1) strengths and, the other half, biowaiver for manufacturing site change (n=2). The decision outcome was 1 application withdraw from the sponsor, 2 denials and 1 approval by ANVISA. All applications were subject to regulatory questions and for the ones denied, the sponsor could not provide the data and justification necessary. The trend of rapid increase in PBBM applications necessitates the exploration of ANVISA current regulations to accommodate this demand and to provide guidance on M&S good practices for industries.

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Study of amikacin POPPK in hospitalized patients with diminished creatinine clearance

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Amikacin (AMK) is an aminoglycoside antibiotic widely prescribed to treat life-threating infections, with concentration-dependent activity and a narrow therapeutic index, which complicates the establishment of an optimal dosing regimen [1-3]. The drug disposition in target populations allows clinicians to optimize drug regimen during therapyM [4]. This study aimed to evaluate population pharmacokinetics (popPK) of patients with diminished creatinine clearance (CrCl) treated with AMK at the University Hospital of Santa Maria (HUSM), Brazil. Peak and trough steady-state blood samples were collected and plasmatic AMK concentration was quantified with liquid chromatography-tandem mass spectrometry [5]. PopPK analysis was performed using MONOLIX™ v.2020R1 (Lixoft, France). Adult patients (n=16) with CrCl < 60mL/min were enrolled in this study. A one-compartment model provides the best results in terms of goodness of fit plot, accuracy of parameter estimation and model stability. The following parameters were estimated by the popPK model: volume of the central compartment (V = 19.6 L; RSE% 20.6); total body clearance (CL = 1.03 L/h; RSE%17.3); standard deviation of the random effects omega_V = 54 %, omega_CL = 63%; a proportional error model was adopted, with parameter being b = 0.32. The parameters estimated in our population are similar with other previously published in the literature [1, 2]. In the present study, our popPK model was able to characterize the PK profile of the patients. Although a further study with more patients should be conducted and covariate effect analyzed in this population. The obtained results would be useful information on the dosing optimization and individualization in this patient's population.

Ethical approval

The study was approved by the University's Research Ethics Committee (CAAE: 83200618.7.0000.5346).

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Physiologically based pharmacokinetic modeling to evaluate the DDI between bupropion and rifampicine in pacients with tuberculosis

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Pharmacological therapy for Tuberculosis (TB) is accomplished reduction transmission of *Mycobacterium tuberculosis* and patient cure. One of the factors to poor prognosis of TB is the tabagism. Bupropion (BUP) is a drug used to treat tabagism [1] and to be successful treatment is required to ensure adequate plasma concentrations of BUP and its metabolite hydroxy bupropion (OHBUP), however when a CYP2B6 inductor rifampicin (RIF) is used together in the TB treatment, a probable interaction would happen. In this sense, a PBPK has been employed to rate drug-drug interactions between BUP and OHBUP and RIP in patients with tabagism and TB. The model was developed using PK-SIM® software using literature reports. The average fold error was between 0.5-2 for all PK parameters evaluated for all drugs. DDI was simulated using virtual population with tabagism and TB, for 3 months, according to Brazilian guidelines [2], and RIF did not influence the BUP and OHBUP concentrations. Then, the DDI modeling has exhibited a significant performance of model to prediction interaction among the RIF, BUP and OHBUP. The obtained results report that concomitant administration of bupropion and hydroxy bupropion supply required concentrations in smoking patients with TB contributing to their prognostics.

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A method to assess model-based bioequivalence

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Model-based bioequivalence (MBBE) encompasses the use of nonlinear mixed effect (NLME) models in bioequivalence (BE) analysis, supporting the estimation of pharmacokinetic endpoints to assess the relative biopharmaceutical performance between drug products. The application of MBBE is being evaluated as a valuable tool to overcome limitations on study design. In turn, specific statistical methods are being developed and compared to the traditional BE approach. Previously, we implemented a MBBE method to estimate the relative bioavailability between two formulations with different release properties (immediate-release vs extended-release) and showed a superior performance versus the traditional approach by overcoming limitations in the sampling design [1]. In this method, concentration-versus-time profiles are initially simulated from an estimated NLME model considering parameter uncertainty and pharmacokinetic metrics (Cmax, AUC) are computed. The CI90% for the test/reference geometric ratio of means for each metric is then obtained through ANOVA on the log-transformed metrics. Here, we compare the performance of this method versus a previously reported method [2] and the traditional BE approach. A two-compartment population pharmacokinetics model including a formulation effect on the bioavailable dose equal to 1.25 was used to estimate the type I error rate of the methods under different scenarios studying the impact of interindividual variability (10,10,5% and 50,50,50% in ka, CL and Vc, respectively), interoccasion variability (0% and 10% in ka, CL and Vc), residual unexplained variability (10 and 20%) and study design (sampling, balanced/unbalanced and number of subjects). MBBE methods showed an appropriate and controlled type I error rate under the evaluated scenarios.

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Mechanistic based PK/PD model describes changes on cortical dopamine concentrations following quetiapine administration to schizophrenia phenotyped rat

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Schizophrenia (SCZ) is a severe mental disorder associated with a dysregulation in brain dopaminergic circuits, which presents a high therapeutic variability [1]. Quetiapine (QTP) is an antipsychotic that increases catecholamine levels as dopamine (DA) in prefrontal cortex, playing a role in SCZ treatment [2]. In the present work, we aimed to develop a pharmacokinetic/pharmacodynamic (PK/PD) model to describe extracellular DA concentrations in naïve and schizophrenic animals. A neurodevelopmental animal model of schizophrenia was implemented previously [3] in Wistar rats offspring, and confirmed with prepulse inhibition (PPI) test. Dopamine baseline and concentrations after QTP 5 mg/kg i.v. *bolus* dosing to the naïve and schizophrenic phenotype (SPR) male and female rats were determined by microdialysis on medial prefrontal cortex (mPFC) using CMA 12 probes (3 mm-CMA, μDialysis®). The quantification of DA in microdialysate was performed by LC-ESI-MS/MS [4]. DA concentrations at baseline and over the course of the study were lower in SPR animals compared to naïve animals with gender related differences as well. The PK/PD model was developed in NONMEM 7.4 (Icon®). The predicted brain unbound levels of QTP were generated from a previous developed semi-mechanistic popPK model [5]. DA concentrations were best described

with a model considering that the QTP-D2 receptor complex inhibits DA post synaptic re-uptake. Our results indicate that the pharmacodynamics of QTP is altered in SCZ, and that the differences seen in the *in vivo* DA profiles between naïve and SPR animals cannot be attributed exclusively to altered pharmacokinetics associated to the disease.

Ethical approval

This study was approved by the Ethics Committee in Animal Use from the Federal University of Rio Grande do Sul (UFRGS/CEUA #31001).

Financial support and acknowledgements

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Acute *Klebsiella pneumoniae* lung infection in rodents decrease ciprofloxacin total clearance

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Therapeutic drug monitoring commonly uses antimicrobials plasma concentrations as surrogate of biophase pharmacologically active concentrations. This study aims to develop a population pharmacokinetic (POPPK) model to characterize ciprofloxacin (CIP) plasma concentrations on acute *Klebsiella pneumoniae* lung infection in rodents. Drug plasma concentrations in healthy rodents were obtained from a previous study [1]. The POPPK model was developed using NONMEM® (version 7.4.3) with FOCE+I and R software was used for graphical visualization. Plasma data from healthy (n = 6) and acutely infected (n = 6) groups following 20 mg/kg i.v. *bolus* dosing, quantified by a validated LC-MS/MS method [2], were described as a three-compartment model with first-order elimination. Interindividual variability was identified on central clearance (22.6%) and on the volume of the second peripheral compartment (14.5%). Infection was added as a covariate on clearance, which showed a 25% reduction on acutely infected group (CL = 0.738 L/h/kg) in comparison to healthy one (CL = 0.991 L/h/kg). The increased CIP plasma concentrations in *K. pneumoniae* acute infection could mislead the clinician to overestimate the antimicrobial lung effect. The real impact of this infection on CIP free lung concentrations remains to be evaluated.

Ethical approval

UFRGS´Ethics Committee in Animal Use #36515.

Financial support and acknowledgements

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External evaluation of single and multi-model methods for model-informed precision dosing of vancomycin in critically ill patients

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Vancomycin is a widely used antibiotic to treat Gram-positive bacterial infections in critically ill patients. The variable pathophysiological condition of these patients directly impacts drug pharmacokinetics, hindering the achievement of successful therapies. Vancomycin AUC-guided dosing through model informed precision dosing (MIPD) is recommended for therapeutic drug monitoring (TDM) [1]. Several vancomycin population pharmacokinetics (PopPK) models have been reported in critically ill patients and could be prospectively implemented; however the external evaluation of their predictive performance is required. In addition to using a single PopPK model, multi-model methods for MIPD: model selection algorithm (MSA) and model averaging algorithm (MAA), have been reported to improve the predictive performance at the individual level [2]. In this work, we explored and compared these approaches by retrospectively evaluating 10 reported PopPK models with vancomycin TDM data from 31 critically ill patients. Relative bias (rBias) and relative root mean squared error (rRMSE) were calculated by a priori prediction and including measured vancomycin concentrations. The *a posteriori* individual AUCs were estimated using best fit single models, MSA and MAA, and the efficacy/safety interpretation were compared versus using trough concentrations (Ct). MSA, MAA, and two single models met the acceptance criteria (rBias<25%) [3]. Multi-model methods achieved better precision. Overall, AUC criteria using MIPD

methods indicated that ~50% of patients achieved safe and efficacious exposure, versus ~20% according to the traditional approach, being more (~50%) the patients with subtherapeutic Ct. In conclusion, as previously reported, the use of Ct would lead to increased vancomycin doses and therefore higher risk of drug-induced toxicity.

Ethical approval

The clinical study was approved by the Institutional Ethics Committee (*Comité Institucional de Ética en Investigación en Salud, CIEIS, Hospital Córdoba, Argentina)* with registration number 346.

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Population pharmacokinetics of tenofovir/emtricitabine among transwomen at HIV risk on oral PrEP and feminizing hormone therapy: Analysis of potential interactions on PrEP

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Introduction: Potential interactions between feminizing hormone therapy (FHT) and preexposure prophylaxis (PrEP) may be a barrier to PrEP use among transwomen (TGW). We aimed to assess the impact of FHT on PrEP pharmacokinetics (PK) among TGW, by population-pharmacokinetic analysis. **Methods:** TGW were assigned to receive PrEP only (noFHT) or FHT (sFHT; oestradiol valerate 2–6mg plus spironolactone 100–300mg) plus PrEP for 12 weeks, after which they could start any FHT. PK assessments occurred at Weeks 12 and 30–48 (plasma samples prior and 0.5, 1, 2, 4, 6, 8, and 24h after dose). Concentration-time data were analyzed using SAEM algorithm on Monolix®. **Results:** The population-pharmacokinetic model was developed using data from Week12. Concentration-time data from 30 participants (noFHT: 12, sFHT: 18) were analyzed (266 observations). A two-compartment model with first-order absorption and lag-time was used to describe tenofovir and emtricitabine PK. In tenofovir model, interindividual variabilities of ka, Vc/F, and CL/F were estimated. Body mass index (BMI) was associated with Vc/F and CL/F; a correlation between these two parameters was also observed. Considering emtricitabine model, interindividual variabilities were attributed to lag-time, ka, CL/F, and CL_2/F. A correlation between CL/F and CL_2/F was observed; age and BMI were associated with emtricitabine CL/F. Discussion: The population-pharmacokinetic models indicated no impact of FHT or oestradiol

levels on tenofovir or emtricitabine PK. The influence of BMI and age on PrEP PK was already reported by previous studies [1,2]. **Conclusions:** Our results indicate no interaction of FHT on tenofovir and emtricitabine PK, further supporting PrEP use among TGW using FHT

Ethical approval

Evandro Chagas National Institute of Infectious Diseases (INI)-FIOCRUZ Institutional Review Board (CAAE: 08405912.9.1001.5262).

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Plasma piperacillin concentrations in Mexican patients from neonatal intensive care unit (a pilot study)

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The physiological differences and the organic immaturity of the neonates, contribute to a high piperacillin pharmacokinetic (PK) variability [1,2]. This affects the clinical efficacy of the antibiotic treatment and increases the probability of developing drug resistance and nephrotoxicity. The present work aims to describe the plasma piperacillin concentrations (CPIP) in Mexican neonates with severe infections on 100 mg/Kg treatment every 8 or 12 hours. A total of 34 CPIP from 15 neonatal intensive care unit (NICU) patients were determined by a liquid chromatography/tandem mass spectrometry (LC/MS/MS). The overall median value (range) postnatal age, gestational age, postmenstrual age, body weight, body-surface area, serum creatinine and glomerular filtration rate (GFR) were 11 (8-27) days, 33.5 (28-40) weeks, 35.2 (30-41.3) weeks, 1815 (995-3190) grams, 0.15 (0.10-0.21) cm^2, 0.47 (0.32-1.05) mg/dL and 34 (14-65) mL/min/1.73 m^2 respectively. Four neonates achieved the PK-PD target defined as CPIP >20 mg/L for more than 75% of the dosing interval (75% fT>MIC). Seven patients were below to the PK-PD target and had CPIP in a range from 2.5 to 16.4 mg/L at 6-or-9 hours post-infusion. Four neonates experienced potential toxicity CPIP>150 mg/L. In patients with life-threatening infection the rapid pathophysiological fluctuations can impact the pharmacokinetics of antibiotics and difficult to achieve the PK-PD target [3]. In this study the dosing regimen was not adequate for some patients. To identify the possible association between the covariates and CPIP it is necessary to develop a PK model for piperacillin in neonates to optimize antibiotic therapy in this special population.

Ethical approval

The present study was approved by the Comité en Ética e Investigación del Hospital Central "Dr. Ignacio Morones Prieto" with the registration code: CONBIOETICA-24-CEI-001-20160227.

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Population pharmacokinetic modeling (popPK) of free and nanoencapsulated meloxicam in male wistar rats

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Meloxicam (MLX) is a non-steroidal anti-inflammatory drug used in the therapy of osteoarthritis, arthritis and muscle discomfort [1]. MLX has high intestinal permeability, however, its low dissolution is a limitation for its use. Thus, the objective of this work was to model PopPK from free and nanoencapsulated MLX. Then, anionic polymeric nanocapsules loaded with MLX (NCM) were formulated by the interfacial deposition method of preformed polymer at a concentration of 1mg/mL and the PK of free MLX and NCM was evaluated in male Wistar rats after iv administration of 5 mg/kg (n=8 animals/group). Blood samples were collected and quantified on HPLC-PDA. The dataset with preclinical observations was transferred to MonolixSuite™ 2020R1 (Simulation Plus, USA), and the final model was two-compartment model with linear elimination, combined error 1. Covariate analysis showed that the NCM affected the Clearance (Cl), the central (V1) and peripheral (V2) Volume of Distribution parameters with positive values of beta. Therefore, from the data modeling, it was possible to observe the nanocapsules containing meloxicam positively influenced Cl, V1 and V2.

Ethical approval

The experimental protocol was approved by the Ethics Committee on the Use of Animals of the UNIPAMPA (030/2021).

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Validation of an LC-ESI-QTOF-MS method to measure cefepime in the plasma and peritoneal fluid of rats

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Introduction: Cefepime (CEF) can be administered in secondary peritonitis to treat sepsis. It is a fourth-generation cephalosporin used in the treatment of severe hospital-acquired infections. **Methods:** Chromatographic separation was performed using a Shimadzu Shim-pack CLC-ODS C_{18} column (250 mm x 4.6 mm), a C_{18} pre column, (4 mm, 5 µm, Phenomenex) and isocratic elution. Gallic acid was used as the internal standard (IS). The mobile phase consisted of (A) ultrapure water and (B) acetonitrile (80:20, v/v) at 0.8 mL/min. Quantification was performed using a mass spectrometer with electrospray ionization in positive mode to monitor ions with m/z 481.1322 (CEF) and m/z 171.0288 (IS). The method was validated employing FDA guidance. The applicability of the method was evaluated in Wistar rats. Plasma and peritoneal microdialysate samples were collected for 4 hours. Pharmacokinetic parameters for CEF were calculated using non-compartmental analysis. Results and discussion: The optimization of the analytical conditions allowed the chromatographic separation of CEF and IS, with retention times of 3.9 min and 4.5 min, respectively. All analytical performance parameters were approved. Pharmacokinetic parameters for CEF were: half-life = 1.10 ± 0.04 and 0.94 ± 0.07 hours; area under the curve = 137.12 ± 25 h.mg/L and 46.53 ± 14.28 h.mg/L for plasma and peritoneal microdialysate, respectively. Conclusion: The technique was validated and successfully applied in a pilot pharmacokinetic study for estimating the free concentration of CEF in the peritoneal microdialysate of Wistar rats for the first time.

Ethical approval

The experimental protocols involving animals were approved by the Ethics Committee on the Use of Animals of the University of Caxias do Sul (007/2020).

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Implementation of therapeutic drug monitoring of 5-fluorouracil in gastrointestinal and colorectal Chilean cancer patients

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Introduction: 5-fluorouracil (5-FU) is the cornerstone of chemotherapy for the treatment of colorectal and gastrointestinal cancer. It has a wide interindividual variability when is dosage based on body surface area (BSA), with 2/3 patients not achieving optimal therapeutic ranges. Therapeutic drug monitoring (TDM) of 5-FU has emerged as a high valuable tool for optimize dosage and improve the safety and efficacy. The aim of this study is to evaluate the clinical and pharmacokinetic impact of 5-FU TDM in Chilean patients with colorectal and gastrointestinal cancer. **Methods:** One-comparment lineal population model implemented in the Abbott base PKS System® software was used for individual pharmacokinetic parameter Bayesian estimates. Plasma concentrations of 5-FU were measured by High-Performance Liquid Chromatography. The Area Under the Curve (AUC) between 20 - 30 mcg x h/mL was considered as the therapeutic objective. Safety was evaluated by comparing severe adverse effect related to BSA or TDM dosage **Results:** Thirty-two patients were recruited with Servicio Salud Valdivia Committee Ethical Approval. Only 28% of the patients dosed by BSA were within therapeutic range, while TDM reached 89% dosage within AUC-objective, with AUC differences between patients up to 4 times. The PK parameters of 5-FU estimated were: volume of distribution (Vd) = 22.1 ± 3.2 L and clearance (Cl) = 218.9 ± 63.8 L/h. Severe adverse effect was more frequent in BSA (4.27%) vs TDM-dosage (3.12%). **Conclusions:** 5-FU shown a high PK variability, while TDM a promissory tool for dose individualization with potential to improve clinical outcomes.

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Biopharmaceutical studies of flavonoids epicatechin and Naringenin

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Flavonoids are widely distributed in herbal species [1] used in traditional medicine, because their many pharmacological effects. Solubility studies clarify biopharmaceutical properties [2]. The aim of this study was to evaluate the experimental equilibrium solubility (ES) of flavonoids in aqueous media to be applied in drug development. The HPLC-UV methodologies for the flavonoids epicatechin and naringenin were developed and validated. The ES studies were performed for compendial media (phosphate buffer pH 6.8; HCl 0.1 M pH 1.2) and biorelevant media (FaSSGF pH 1.6 and FaSSIF pH 6.5). The theoretical solubility of each flavonoid was assumed added plus 30% of the amount of the standard to guarantee the excess of solute. The shake-flask technique was performed at 37 °C and 250 rpm until equilibrium solubility to be achieve [3]. The samples were centrifuged, and the supernatant quantified. For epicatechin, the theoretical solubility is 5.59 mg/mL for acidic pH, 5.73 mg/mL for neutral pH, and the experimental solubility in phosphate buffer and HCl 0.1 M were 6.81 mg/mL and 6.47 mg/mL, respectively. In FaSSGF and FASSIF the results were 6.77 mg/mL and 6.51 mg/mL, respectively. The naringenin was not stable in acidic media, then, only neutral pH media studies were performed. The theoretical solubility is 0.79 mg/ml for a pH of 7.4 and the experimental solubilities were 1.28 mg/mL for phosphate buffer and 0.31 mg/mL for FaSSIF. The experimental solubility showed higher values compared to theoretical. The accuracy on aqueous solubility of natural compounds is crucial to guide drug development and understanding of pharmacokinetic profile.

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Pharmacometric study of neuroepo in a non-human primate model *Macaca fascicularis*

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Erythropoietin (Epo) is a glycosylated hormone considered the main growth factor in erythroid cells with cytoprotective action on non-hematopoietic tissues such as central nervous system (CNS).[1] NeuroEPO, is a nasal pharmaceutical solution of recombinant Epo with low sialic acid with proven neuroprotective effect at the preclinical and clinical level. [2, 3] A single dose of intranasal NeuroEPO (5750 IU* kg^{-1}) was administered to a population of non-human primates (*macaca fascicularis*) and the C(EPO) vs t profile in plasma of peripheral venous blood and lumbar cerebrospinal fluid (LCF) was determined by sparse sampling. A non-compartmental pharmacokinetic analysis with Phoenix-Winnonlin, 8.3.4.295, (Certara) led to drug targeting efficiency (> 100%) and a direct transport percentage (90,47%) that supported the high NeuroEPO biodistribution in CNS involving nerve pathways. A turnover model accounting for endogenous production in LCF and exogenous dosing was developed using non-linear-mixed-effects models (NONMEM7.4). The inhibitory feedback on Epo synthesis significantly improved the model ($p<0.005$). Four fixed effects were estimated with FOCEI, namely distribution volume ($V=3760\pm481$ mL), endogenous synthesis rate ($Ksyn=53.4\pm4.50$ IU/h), elimination constant ($Kout=3.60\pm0.25$ h^{-1}) and Epo concentration leading to a 50% inhibition of Ksyn ($IC50=0.00241\pm0.00048$ IU/mL). The absorption rate constant was fixed ($Ka=4$ h^{-1}). Interindividual variability was included on V and residual error was modeled as proportional. The stability of the model was proved by means of a non-parametric bootstrap method. Good agreement between model predictions

and observed concentrations was found and vpc confirmed the predictive capability of the model. A mPBPK, is needed to leverage the pharmacokinetics of NeuroEPO in CNS.

Ethical approval

The study was designed and conducted in accordance with international ethical criteria related to non-clinical research in non-human primates and was approved by the corresponding Ethics Committee of the Center for Laboratory Animal Studies.

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